United States Environmental Protection Agency

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title:	Methods development for ultrasound monitoring of prenatal development in pregnant rats.
LAPR Number:	17-09-003
Principal Investigator	Exemption 6
Author of this Document:	Exemption 6 /RTP/USEPA/US
Date Originated:	09/16/2014
LAPR Expiration Date:	09/30/2017
Agenda Date:	09/24/2014
Date Approved:	10/01/2014
Date Closed:	09/12/2017

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6 /RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	10/01/2014	DMR	
	Exemption 6 Exemption 6 Exemption 6 /RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	10/01/2014	Designated Member Reviewer	

Administrative Information

1. Project Title (no abbreviations, include species):
Methods development for ultrasound monitoring of prenatal development in pregnant rats.

Is this a continuing study with a previously approved LAPR?
No

2. What is the Intramural Research Protocol (IRP) number covering this project?
IRP: NHEERL-RTP/TAD/ETB/Exempti Exempti Exempti/2014-001-r0
Safe and Sustainable Water Resources (SSWR) Task 2.2.D: Integrated Assessment and Reduction of Contaminant Risks

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6 *Lotus Notes Address* Exemption 6 Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US	TAD *Branch* ETB	MD

4. Alternate Contact:

Alternate Contact	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6 *Lotus Notes Address* Exemption 6 Exemption 6 Exemption 6 Exemption 6 Exemption 6 TP/USEPA/US	TAD *Branch* ETB	MD 67

SECTION A - Description of Project

1. Study objectives, presented in non-technical language such that it is understandable by non-scientific persons, including how the study addresses health protection. If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.
Conventional developmental toxicity studies in rodents are required by regulatory agencies (e.g., FDA, EPA, and internationally) and aid in risk assessment of pharmaceuticals and environmental pollutants. These studies involve examination of full-term fetuses or neonates for malformations and structural variations. Examination of the rodent fetal heart is particularly challenging and requires great skill and expertise. Several different dissection

and sectioning techniques have been employed, but each approach has advantages and disadvanteges. One important disadvantage common to all conventional heart examination techniques is that the act of conducting the examination destroys the organ structure, often making it difficult/impossible for a subsequent examiner or consultant to verify findings. Furthermore, although these techniques evaluate structure, they do not evaluate cardiac function.

Here, we hope to use ultrasound to evaluate fetal hearts; this state-of-the-art approach offers several important advantages over conventional heart examination techniques. This technique will allow us to non-invasively monitor cardiac function as well as cardiac structure at multiple time-points during fetal development. Also, digital records of the ultrasound examination will allow confirmation of findings by subsequent examiners. Thus, this approach promises to provide a more sensitve evaluation of cardiac abnormalities, will be valuable for evaluating toxicants suspected of affecting heart development, and will refine the risk assessment of such chemicals.

Prior to using this state-of-the-art technique, we need hands-on experience to learn the instrument's capabilities and learn to conduct ultrasound fetal examinations at various time points during gestation. The purpose of this LAPR is to develop these techniques in preparation for applying it in future developmental toxicity studies.

Our developmental toxicity studies typically use two different strains of rat: Sprague-Dawley and F344. These strains react differently to handling, restraint, and potentially anesthesia (for restraint); thus, we propose methods development with both of these strains.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

In vivo testing is an essential part of assessing reproductive and developmental toxicity hazard in that the intact live animal is the only test system available that incorporates the complex maternal-embryonic interactions of development.

b. Justify the species requested:

Rats have been used extensively in developmental toxicology because of their size, ease of care, fecundity, and large historical database. Our work will gain insights from, and add to, the large historical database of reproductive and developmental toxicity research in this species.

3. How was it determined that this study is not unnecessary duplication?

No existing or published study is sufficient to provide hands-on training or learn instrument capabilities.

SECTION B - In Vivo Procedures

1. Briefly describe experimental design. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

Timed-pregnant rats will be purchased and, at various time-points during gestation, subjected to abdominal ultrasound (via a wand/probe) in order to examine embryos/fetuses in utero.

The dams will need to be immobile during the ultrasound evaluations. The instrument vendor recommends isoflurane anesthesia for animal restraint, and we expect that this will be reliable, and especially helpful for our initial hands-on training with the instrumentation. However, we are concerned that in future developmental toxicity studies, anesthesia could potentially interact with the toxicant being studied and confound the results of the study. Thus, we will also attempt using gentle hand-held restraint without anesthesia to conduct ultrasound examinations.

Dams will be euthanized prior to parturition.

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.

We request 20 timed-pregnant females (10 Sprague-Dawley, 10 F344) rats for development of ultrasound examination procedures. Animals will be ordered incrementally (e.g., two or four at a time), to allow training opportunities with as few animals as possible.

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	20	
D) Potential pain/distress relieved by appropriate measures:		
E) Unrelieved pain/distress:		

4. For tracking purposes, please check if this LAPR includes any of the following:

- ☒ Restraint (>15 Minutes)
- ☐ Food and/or water restriction (>6 Hours)
- ☐ Survival surgery
- ☐ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):

b. Survival Blood Collections (method, volume, frequency):

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):

Ultrasound Echocardiography: Fetal cardiac examinations will be conducted via high-frequency echo using a VisualSonics Vevo 2100 ultrasound system in room Exemption 6 Exemption 6 Exemption 6.

For restraint under anesthesia, dams will be placed in a sealed chamber and anesthetized with 2-5% Isoflurane delivered in 100% O2 at 0.8-1 L/min. We will then move the dam to a heated procedure table where anesthesia will be maintained with 1-4% Isoflurane (100% O2 @ 0.8-1 L/min) via a nose cone. The eyes will be coated with eye lubricant to prevent drying of the eyes during the procedure. Each paw will be gently taped to ECG electrodes coated with electrode gel (to monitor heart rate and respiratory rate) and body temperature will be monitored with a rectal probe. Nair gel will be used to remove fur from the imaging location (abdomen). The application area will be washed to remove any residual Nair from the skin. Prewarmed ultrasound gel will be applied to the abdomen and the HF Echo transducer will be used to noninvasively record video loops and collect data on as many conceptuses as practical. Potential endpoints include blood flow, heart rate, contractile motion of the heart, and septation. After the measurements are made, the dam will be gently and carefully cleaned of all transducer gel, removed from anesthesia, kept on a heating pad until ambulatory, and placed in her home cage. Anesthesia restraint may last up to approximately 45 minutes.

For restraint without anesthesia, the dam will be held by one of the research staff (listed in Section E) while another research staff member will collect measurements. Nair gel will be applied to the abdomen for fur removal, and the application area will be washed to remove any residual Nair from the skin. Prewarmed ultrasound gel will be applied to the abdomen and the HF Echo transducer will be used to noninvasively record video loops and collect data on as many conceptuses as practical. Potential endpoints include blood flow, heart rate, contractile motion of the heart, and septation. After the measurements are made, the dam will be gently and carefully cleaned of all transducer gel, and returned to her cage.

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:

Restraint for ultrasound measurements will be accomplished by isoflurane anesthesia (see B5c) or by hand-held restraint without anesthesia.

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):

NA

f. Describe how animals will be monitored (e.g., frequency of observations, by whom):

Animals will be monitored by laboratory staff (listed in Section G).

During anesthesia, the dam's heart rate, respiratory rate, and body temperature will be monitored continuously.

On regular work days, dams will be handled daily by laboratory staff (listed in Section G) throughout gestation; during this time, animals will be monitored for general health. (Daily handling will help tame the animals and facilitate restraint without anesthesia.)

Dams will be monitored several times per day for signs of parturition starting on GD 20. (Parturition normally does not begin until the afternoon of GD 21 or later.)

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):

b. Survival Blood Collection (method, volume, frequency):

c. Testing methods:

d. Restrictions placed on the animals' basic needs (e.g., food and/or water deprivation, light cycles). Provide details regarding the length of deprivation:

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:

7. Surgical Category D and E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:

b. Anesthetic regimen (drugs, dosages, volume, and route of administration). The use of paralytic or neuromuscular blocking agents without anesthesia is prohibited:

c. Postoperative care (thermal support, special feeding, frequency and duration of monitoring, responsible personnel, removal of sutures/staples):

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):

e. Will any animals be subject to more than one major surgical survival procedures?
◯ Yes ◯ No

f. Identify any surgical procedures performed at other institutions or by vendors:

8. Humane interventions (for treatments/procedures in all categories).

a. Describe actions to be taken in the event of expected or unexpected deleterious effects from procedures or chemical exposures.
Animals will not be treated with toxicants. However, if animals show symptoms of physical injury, dystocia, rough hair coat, or deteriorating body condition we will euthanize or otherwise follow AV recommendations.

b. State criteria for determining temporary or permanent removal of animals from the study.
Iif animals show symptoms of physical injury, rough hair coat, or deteriorating body condition we will euthanize or otherwise follow AV recommendations. If animals show signs of parturition or dystocia, we will

euthanize immediately.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study: 20

b. Animals to be transferred from another LAPR:

LAPR Number that is the source of this transfer:

c. Animals to be transferred from another source:

d. Offspring produced onsite (used for data collection and/or weaned):

e. TOTAL NUMBER of animals for duration of the LAPR 20

2. Species (limited to one per LAPR): Rat(s)

3. Strain: Sprague Dawley, F344

Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

none

4. Sources of animals:

Charles River Laboratories, Harlan Sprague Dawley

5. Provide room numbers where various procedures will be performed on animals:

Exemption 6

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

no ***Room Numbers:***

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)

NA

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.

Ultrasound examinations may be conducted less than 3 days after arrival to allow examinations at appropriate times during gestation.

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

none

10. Housing and Enrichment.

The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.

Dams will be pair-housd.
Heat-treated pine shavings will be used as bedding.
Enviro-dri will be provided.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.
Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.

Isoflurane (pharmaceutical grade): Maximum concentration is 3%. LC50 rat, inhalation = 15,300 ppm. Isoflurane is considered a "potentially hazardous substance" but does not require an HSRP. Isoflurane will be used in the chemical safety hood in Exemption 6.

2. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in this LAPR, and provide:

a. Information to assure that such material is pathogen-free

b. A statement regarding any safety precautions necessary for handling the material.

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:
Exemption 6, student, animal handling, Received IACUC-required training. He will be trained/supervised by Exemption 6.

Hint: *The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).*

NAME	ROLE	SPECIFIC	RELEVANT TRAINING

		RESPONSIBILITY	
Exemption 6	Principal Investigator	Study design; animal handling, ultrasound examinations, cervical dislocation, Category C procedures	~30 years experience. Proficient in cervical dislocation, including rats >200 g. Completed IACUC-required training.
Exemption 6	Technical Staff	animal handling, ultrasound examinations, cervical dislocation, Category C procedures	~30 years experience. Proficient in cervical dislocation, including rats >200 g. Completed IACUC-required training.
Exemption 6	Associate Principal Investigator	Training/consultant for ultrasound examinations	>15 years of experience in use of laboratory animals; > 7 years surgical procedures and physiological monitoring; completed NHEERL animal use/care training
RTP-NHEERL	Tech Support	Category C Procedures	EPA IACUC Trained

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year

2. Breeding protocols and recordkeeping

3. Methods for monitoring genetic stability

4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

Dams will be euthanized on gestation day 20 or 21, before parturition.

If cesarean delivery of fetuses is warranted (e.g., to confirm malformations detected by ultrasound), fetuses will be euthanized by decapitation; extra scissors will be available.

2. Describe the euthanasia techniques:

Method(s): Cervical dislocation of dams, Decapitation of fetuses
Agent(s):
Dose (mg/kg):
Volume:
Route:

Source(s) of information used to select the above agents/methods:

_ Personal Experience

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition
NHEERL Best Practices: Fetal and Neonatal Euthanasia

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the 2007 American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).

Cervical dislocation of rats >200g: The 2013 AVMA Guidelines for the Euthanasia of Animals recommends cervical dislocation as a method of euthanasia for rats weighing <200g when performed by individuals with a demonstrated
high degree of technical proficiency. It also states that the large muscle mass in the cervical region of heavy rats makes manual cervical dislocation physically more difficult. The Guideline's 200-g weight limit is flawed for two important reasons: 1) The additional weight acquired during pregnancy or lactation has little, if any, influence on the muscle mass of the neck. (E.g., our F344 rats typically weigh 200-250 g during late pregnancy, but their nongravid weights are <180g). 2) The technique for performing cervical dislocation described by the AVMA Guidelines is appropriate for mice, but it is an inferior technique for rats. Rather than using the thumb and index finger, the preferred technique involves placing the index and middle fingers on either side of the animal's neck (from the dorsal aspect with the palm facing rostrally). Unlike the Guideline's method, this method IS appropriate for heavier animals and is NOT physically more difficult. The Principal Investigator of this project has >25 years experience performing this technique on nongravid rats weighing >350g and pregnant or lactating rats weighing >500g.

4. Describe how death is to be confirmed.

Vital organ section, Prolonged absence of breathing

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.

Euthanized as above

The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?

● Yes ○ No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.
2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.
3. The proposed research using animals does not unnecessarilty duplicate any previous experimentation.
4. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
5. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
7. Individuals from outside of EPA who are collaborating on this project, and who conduct related experimentation on EPA procured or bred animals in their respective Institutions, have the equivalent of a current IACUC approved LAPR at their respective Institutions.
8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	09/17/2014

Submitted: 09/17/2014

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	***Approval Date***	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	09/17/2014	Exemption 6	TAD	MD
		Lotus Notes Address	***Branch***	***Submitted to Branch Chief for Approval***
	by Exemption 6 Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	Exemption 6 Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	ETB	09/17/2014 11:34 AM

ATTACHMENTS

17-09-003 PI resp.pdf

Actions

First Update notification sent: 07/31/2015
Second Update notification sent:
First 2nd Annual notification sent:
07/27/2016
Second 2nd Annual notification sent:

1st Expiration notification sent: 08/07/2017
2nd Expiration notification sent: 09/01/2017

History Log: